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Abbreviations

AKME	Adjusted Kaplan-Meier estimator
CDC-XM	Complement-dependent cytotoxicity crossmatch
DBD	Donation after brain death
DCD	Donation after cardiac death
DSA	Donor-specific HLA antibodies
HLA-Abs	HLA antibodies
FCXM	Flow cytometry crossmatch
MFI	Median fluorescence intensity
NOTR	Netherlands Organ Transplant Registry
PRA	Panel reactive antibody
SAB	Single antigen bead
Tx	Transplantation

Abstract

The presence of donor-specific anti-HLA antibodies (DSA) is associated with increased risk of graft failure after kidney transplantation. We hypothesized that DSA against HLA class-I, -II or both indicate a different risk for graft loss between deceased- and living-donor transplantation. In this study we investigated the impact of pretransplant DSA, assessed using single antigen bead assays, on long-term graft survival in 3237 deceased- and 1487 living-donor kidney transplantations with a negative complement-dependent crossmatch. In living-donor transplantations, we found a limited effect on graft survival of DSA against class-I or -II antigens after transplantation. Class-I and -II DSA combined resulted in decreased 10-year graft survival (84% to 75%). In contrast, after deceased-donor transplantation, patients with class-I or class-II DSA had a 10-year graft survival of 59% and 60% respectively, both significantly lower compared to patients without DSA (76%). The combination of class-I and -II DSA resulted in a 10-year survival of 54% in deceased-donor transplantations. In conclusion, class-I and -II DSA are a clear risk factor for graft loss in deceased-donor transplantations, while in living-donor transplantations class-I and -II DSA seem to be associated with an increased risk for graft failure but this could not be assessed due to their low prevalence.

Introduction

Kidney transplantation is the best treatment option for patients with end-stage renal disease (1). The discovery and development of potent immunosuppressive drugs that are able to prevent or treat rejection has greatly improved short-term graft survival rates over the past 50 years (2). Despite these advances, various large registries show graft failure rates of approximately 10% within the first year after transplantation, increasing to up to 40% at 10 years after transplantation. To further improve the outcome of kidney transplantation there is a clear need for parameters that enable risk stratification for graft failure (2–7)

In the Eurotransplant region the presence of donor-specific HLA antibodies against a potential donor kidney causing complement-mediated lysis (8) is considered a contraindication for transplantation . These antibodies can be detected with the complement-dependent cytotoxicity crossmatch assay (CDC-XM), which has been the gold standard since 1969. With the more recently developed single antigen bead (SAB) assays, DSA can be detected with increased sensitivity and specificity (9), but the relation between these SAB-detected antibodies and clinical outcome is still unclear (10–14). The presence of SAB-detected antibodies that do not cause a positive CDC-XM is not a contraindication for transplantation, but may indicate an increased immunological risk for rejection and allograft loss (15).

It is well known that graft survival rates in patients who received a living-donor kidney are higher compared to that in recipients of a deceased-donor kidney (16). While recent large cohort studies on the impact of DSA on graft survival have mainly focused on deceased-donor transplantations (17–19), the

effect of SAB-detected DSA on living-donor transplantations has not been studied in large cohorts. In a Japanese single center study 324 living-donor kidney transplant recipients were analyzed to investigate the outcome of the 92 kidney transplant recipients with DSA in combination with anti-blood type, anti-HLA antibodies or both which all were desensitized prior to transplantation (20). They reported no significant difference in graft survival of the different groups compared to no DSA group at 1 and 5 years after transplantation. As far as we could find, there we no large cohorts describing the effect of pretransplant DSA with negative CDC-XM in exclusively living-donor kidney transplantations without desensitization treatment. Orandi et al (21) studied the outcomes of incompatible living donor kidney transplantations based on the risk determined using the SAB-assay, flow cytometry crossmatch (FCXM) or CDC-XM and found that in case of positive SAB-assay and a negative FCXM patients (n=185) which were desensitized had similar graft survival as compared to a large group of compatible patients (n=9669), while patients with a positive FCXM (n=536) or a positive CDC-XM (n=304) experienced an increased risk of graft loss. Another study about living donor transplantations performed after a positive FCXM (n=41) reported that the long-term survival was worse in desensitized recipients compared to matched recipients with a negative FCXM (n=41) (22).

In a single center study, including both living and deceased-donor transplantations, it was shown that patients with combined pretransplant HLA class-I and -II DSA had an increased risk of graft loss (23). As part of a Dutch national Profiling Consortium of Antibody Repertoire and Effector functions (PROCARE), all kidney transplantations performed in the Netherlands between 1995-2006 were evaluated retrospectively (24). This cohort was selected for several reasons: allocation or choice of immunosuppressive therapy was not influence by results of SAB-defined DSA, patients had at least 10 years of follow-up and relatively modern immunosuppression. We analyzed whether SAB-detected DSA against HLA class-I and/or -II influence long-term graft survival in deceased- and living-donor kidney transplantations.

Materials and Methods

Patients, sera and clinical data

This multicenter study included all 6097 kidney transplantations performed between January 1995 and December 2005 in all Dutch transplant centers. Patients were primarily Caucasian. In all cases, the T-cell CDC-XM with current and historic highest sera was negative. Historic cytotoxic HLA antibodies were assigned as unacceptable for allocation in Eurotransplant region. Bead assay defined DSA were not considered as risk factor in the matching procedure at that time therefore had no influence in immunosuppressive treatment. Informed consent for data collection and use of leftover sera was obtained from all subjects. Patients and donors investigated were predominantly Caucasians. The use of sera and experimental protocols was approved by the Research Ethics Committee for Biobanks and the Medical

Ethics Committee of the University Medical Center Utrecht. Moreover, this study was performed in accordance with the FEDERA Code of Conduct.

We obtained baseline and clinical follow-up transplantation data from the Netherlands Organ Transplant Registry (NOTR), which was over 95% complete at time of this study. Clinical follow-up was recorded at 3 months, 12 months, and yearly thereafter for at least 10 years. The primary endpoint of the study is graft failure, defined as loss of kidney function when the patient returns to dialysis or receives a retransplant. In the analysis of death-censored graft failure, recipients who died with a functioning graft were censored at the time of death.

Pretransplant patient sera could be collected from 4787 (78%) transplantations of 4585 patients (some patients underwent more than one transplantation). 17 transplantations were lost to follow-up (NOTR) and 46 transplantations were excluded because the kidney failed during surgery or shortly thereafter due to technical non-immunological problems. 4724 transplantations were included in the analysis.

Detection and definition of donor-specific HLA antibodies

The presence of HLA antibodies (HLA-Abs) in the pretransplant sera, used for pretransplant crossmatch, was assessed retrospectively in one central laboratory as described previously (25). In brief, sera were first tested for the presence of HLA class-I and class-II antibodies using Lifecodes LifeScreen Deluxe (Immucor Transplant Diagnostics, Stamford, CT). Subsequently the sera positive for HLA class-I and/or class-II were analyzed using Lifecodes SAB assay class-I and/or -II kits (Immucor Transplant Diagnostics) to determine the exact specificity of the HLA antibodies. The LABScan 100 flow analyzer (One Lambda, Canoga Park, CA) was used for data acquisition. Bead positivity was defined according to manufacturer's instructions, requiring a minimum signal to background ratio to be reached (described in (25)), which leads to virtually identical results as when taking an absolute MFI cutoff of 750. The presence of SAB-DSA was assigned by comparing the SAB-HLA-A/B/DR/DQ antibody specificities on serological level with the split level HLA typing of the donor. For 70 antigens (47 of which HLA-DQ) in 64 transplantations we only had broad level typing information at our disposal and could not determine whether antibodies against some of the possible splits were DSA. These donor antigen-recipient antibody combinations were not considered as DSA in the analyses in this manuscript. If we would assign all these antigens as DSA, similar results and the same conclusions were obtained (data not shown).

Statistical analysis

Differences in patient, donor and transplant characteristics between the DSA-positive and -negative group were assessed by the Chi-square test for categorical variables and Mann-Whitney U test for continuous variables. Death-censored graft survival was assessed using the adjusted Kaplan-Meier estimator (AKME) based on inverse probability weighting (IPW) (26). The following covariates were considered for adjustment: recipient and donor age, recipient and donor sex, year of transplant, type of donor, cold

ischemia time, retransplant, graft function, IL-2 receptor blocker, Number of HLA-A/B/DR mismatches, transplant and highest %-PRA. We adjusted for recipient age (quadratic) and donor age (quadratic), donor type (living or deceased; for the total cohort only), cold ischemia time (for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic) and induction therapy with IL-2 receptor blocker (Figure S1). The other covariates were not used for various reasons motivated in the supplemental document. Hazard ratios and confidence intervals were derived using multivariable Cox regression. Validity of Cox model assumptions were verified by evaluating uncorrected Kaplan-Meier, (cumulative) Martingale residual and Schoenfeld residual plots. Various covariates, specified in the supplemental document, were used in both the AKME and Cox regression, to adjust for confounding. 226 missing cold ischemia times were imputed using Markov chain Monte Carlo (MCMC) single imputation, no additional values were missing. Statistical analyses were performed with R (version 3.2.2) and SAS (version 9.4; SAS Institute, Cary, NC) software.

Results

Baseline characteristics

Patient, donor and transplant characteristics stratified according to the presence of pretransplant DSA are summarized in Table 1. 567/4724 (12%) patients had pretransplant DSA. The mean age at transplantation was significantly lower in recipients with DSA. The DSA group contained a higher proportion of female recipients (59% versus 38%) and panel reactive antibody (PRA) values determined with CDC were clearly related to the presence of DSA. Additionally, there were significantly more retransplantations in the DSA (47.6%) group. In 33% of the transplantations without DSA, the kidney was donated by a living donor, whereas 24% of the transplantations with preformed DSA had living donors. Most patients initially received a triple immunosuppressive regimen consisting of steroids, cyclosporine or tacrolimus, and mycophenolate mofetil or azathioprine. 26% of the patients received induction therapy, either with a T-cell depleting antibody (4%) or an IL-2 receptor blocking antibody (22%). Minimal follow-up time was 10 years after transplantation.

Impact of pretransplant DSA on long-term graft survival

Using SAB-assays we determined the presence of antibodies against HLA-A/B/C/DR/DR51-53/DQ/DP antigens, either donor-specific or not. As shown in a Venn diagram (Figure 1A), in 3269/4724 (69%) transplantations the recipients had no pretransplant antibodies against HLA-A/B/DR/DQ antigens. The combination of anti-HLA-A and anti-HLA-B antibodies (without anti-HLA-DR/DQ antibodies) was relatively frequent (311/4724=7%), as was the combination of antibodies against all 4 antigens (254/4724=5%). Antibodies against a single HLA molecule were most frequent for HLA-B and -DQ. The prevalence of antibodies exclusively directed against HLA-C, -DR51-53, or -DP was low in our cohort with 4, 13, and 19

positive sera, respectively (Table S1). Donor-specific antibody prevalence against the donor HLA-loci A, B, DR, and DQ is depicted in Figure 1B. In 4157/4724 (88%) of the kidney transplantations, recipients harbored no pretransplant DSA against these antigens.

The AKME showed a 10-year death-censored graft survival of 78% (95% confidence interval (CI) 77%-80%) for the 4157 patients without, and 66% (95% CI 64%-67%) for the 567 patients with DSA in pretransplant serum (Figure 2A). The multivariable analysis, also adjusted for the same covariables, showed that the presence of DSA was associated with a higher risk of graft failure (Table 2; Hazard ratio (HR): 1.77, 95% CI 1.51-2.08).

As our cohort contains a relatively high proportion of living donors, we analyzed the impact of DSA on long-term graft survival according to donor status. For the living-donor transplantations (N=1487), there was only a limited and non-significant relation between pretransplant DSA and 10-year death-censored graft survival, which was 78% and 84% for patients with and without DSA, respectively (Figure 2B,p=0.07). For the deceased-donor transplantations (N=3237), the 10-year death-censored graft survival was 60% and 76% for patients with and without DSA, respectively (Figure 2C, p<0.0001), demonstrating a clear adverse effect of pretransplant DSA.

These findings were confirmed in a multivariable analysis (Table 2), where the presence of pretransplant DSA had no significant influence on graft survival in living-donor transplantations (HR: 1.42, 95% CI 0.95-2.10). In contrast, the presence of DSA was significantly associated with a higher risk of graft failure after deceased-donor transplantations (HR: 1.84, 95% CI 1.55-2.19). In table 3 the patient, donor and transplant characteristics of living (N=1487) and deceased-donor (N=3237) transplantations are summarized. In addition, the characteristics for living- and deceased-donor transplantation were further subdivided for transplantations with DSA (Table S2), for the DSA class I only groups (Table S3), for the DSA class II only groups (Table S4) and for the DSA class I and II groups (Table S5).

Impact of pretransplant DSA on early and late graft failure

The effect of pretransplant DSA on graft survival was already evident early after transplantation (Figures 3A-B). In living-donor transplantations, 1-year death-censored graft survival for patients with and without DSA was 94% and 96% (Figure 3A), respectively (Table 2; HR: 1.69, 95% CI 0.76-3.77). For deceased-donor transplants, we found a similar effect (Figure 3B; HR: 1.72, 95% CI 1.31-2.27).

In order to examine whether pretransplant DSA also affect the risk of late graft failure, we separately analyzed the graft survival of the patients with surviving grafts at 1 year after transplantation. To this end, 70 (for living-donor) and 403 (for deceased-donor) grafts that failed or were censored in the first year were excluded and graft survival analysis was restarted at 100% (Figures 3C-D). For living-donor transplantations, a modest, non-significant, effect of DSA was found, with 82% and 87% 10-year death-censored graft survival for those with and without DSA, respectively (HR: 1.35, 95% CI 0.86-2.12). For deceased-donor transplantations however, an increased risk of graft failure (HR: 1.95, 95% CI 1.56-2.44) remained with a 10-year death-censored graft survival of 70%, compared to 83% for

transplantations in patients without DSA. Similar results were found when an early graft failure cutoff of 3 months was used instead of 1 year (data not shown). These data indicate the presence of a short- and long-term effect of DSA on graft survival in deceased-donor transplantations. For living-donor transplantations no significant effect was found.

Effect of pretransplant HLA class-I and -II DSA on graft survival

Next, we investigated the effects of donor-specific HLA class-I (A/B) and HLA class-II (DR/DQ) antibodies on kidney graft survival within the living-donor transplantations separately. We found no effect on graft survival within 1 year after transplantation of pretransplant DSA against either class-I or -II antigens only, and only a limited effect of 4% and 5% on the 10-year graft survival respectively (Figure 3E; Table 2; HR 1.35, 95% CI 0.86-2.12). In contrast, in deceased-donor transplantation, the isolated presence of either class-I or class-II DSA was clearly associated with an increased risk of graft failure (HR: 1.93, 95% CI 1.51-2.48; HR: 1.74, 95% CI 1.36-2.24, respectively) with a 10-year death-censored graft survival of 60% and 61% respectively, compared to 76% in transplantations with DSA negative recipients (Figure 3F).

The combined presence of class-I and -II DSA resulted in the poorest graft survival for both donor types, with a decrease from 84% to 75% 10 year after transplantation for living-donor grafts (HR: 2.84, 95% CI 1.05-7.69) and a decrease from 76% to 54% for deceased-donor grafts (HR: 1.90, 95% CI 1.25-2.88).

Discussion

In this multicenter study we found a limited effect of pretransplant SAB-defined DSA on graft failure in living-donor transplantations. In contrast, pretransplant SAB-defined DSA are a clear risk factor for graft loss in deceased-donor transplantations with a negative CDC-XM. Further subdivision of the DSA in deceased-donor transplantations revealed that DSA against either class-I or -II did constitute a significant risk factor for graft loss and pretransplant DSA against both HLA class-I and -II resulted in the poorest death-censored graft survival. In living-donor transplantations, the combination of class-I and –II DSA seem to be associated with an increased risk for graft failure but this could not be assessed due to their low prevalence.

Recently published studies on pretransplant DSA focused mainly on deceased-donor transplantations, as these are most prevalent in, for example, France, Germany, and the USA (17–19). Studies on living-donor transplantation are scarce; in a single-center study, where 324 living-donor transplantations were analyzed, no significant difference in 5-year graft survival of patients with DSA was found as compared to patients with anti-blood type antibody, anti-HLA-Abs, or no DSA (20). Mohan et al (19) reported a meta-analysis of DSA detected with amongst others SAB assays, and calculated an increased risk for graft failure in the presence of SAB-DSA with negative CDC-XM, similar to our study. The effect of DSA in living-donor transplantation has not been investigated so far in large cohorts. In the

Netherlands, currently more than half of the kidney transplantations are performed with kidneys from living donors, while the 31% in the cohort from 1995-2006 provides us with the unique means to study a cohort of 1487 living-donor transplantations.

The time period of 1995-2006 was specifically chosen as it ensures sufficient follow-up with immunosuppressive treatment currently still used, without bias due to results from SAB assays in pretransplant risk assessment, patient/donor selection or guidance of immunosuppressive treatment after transplantation. As there is no consensus regarding the MFI cutoff that should be used to determine positivity using SAB assays, we defined positivity based on manufacturers' instructions using a signal to noise ratio. Using different MFI cutoffs for DSA positivity resulted in comparable effects on long-term graft survival (Figure S2). DSA Class I and II positive transplantations have a considerably higher average number of DSA, average maximum MFI of DSA and average cumulative MFI of DSA (Table S7b-d). The higher 'strength' of DSA as expressed by these three parameters could (partly) explain the worse graft survival of this group. DSA Class I and II positivity however provides a better risk classifier than we were able to construct from three DSA strength parameters in this study. Other studies have shown that DSA assessments using MFI alone may not be sufficient for assessing the potential risk for graft damage and decreased survival. The use of multiple assays such as Flow-XM (21,27) , C1q-SAB assays (17), C3d-SAB assays (28) or IgG-subclass analysis (29). Our cohort includes 63 patients participating in the Eurotransplant Acceptable Mismatch program with a HLA antibody profile based on CDC, in some cases supplemented with solid phase assays (30). Inclusion of this patient group did not induce bias as we observed no major impact on our conclusions if we would exclude these patients from our cohort (data not shown). We excluded 46 transplantations because the kidney failed during surgery or shortly thereafter due to technical non-immunological problems. The impact of inclusion and exclusion of these patients on graft survival was equal for both groups (DSA versus no DSA).

Our study has a few limitations. As we collected sera retrospectively we were able to collect 78% in total. We are mainly missing sera from older transplantations of four centers, while from the other three centers we collected over 90%. Limited information was available on rejection and donor organ quality and we do not have information on post-transplant (*de novo*) DSA formation. As this is a retrospective cohort of kidney transplantations between 1995 and 2005, the registration of rejections was limited. We have only information whether patients were treated for a rejection including the date of rejection and, if performed, the date of biopsy. At that time biopsies were not always performed and therefore some of the registered rejections might not have been actual rejections. On the other hand, rejections might have been missed or not registered. Others have shown that in living-donor transplantations DSA was an important predictor of antibody-mediated rejection, while this was not the case for graft failure (31). Using the limited rejection data that we had, we observed that patients with pretransplant DSA had a higher incidence of rejection in living- as well as deceased-donor transplantations (data not shown).

For post-transplant DSA determined using SAB assays it was already shown that it has an adverse effect on graft survival (18). For our cohort we can only assess the potential for confounding by *de novo*

DSA via the average number of HLA mismatches. As the DSA positive groups have fewer mismatches for all HLA-loci (Table S9), we expect to be underestimating rather than overestimating the effect of pretransplant DSA in that respect. We also find fewer mismatches for all loci in deceased-donor transplantations. In addition, the difference in impact of DSA on graft survival between living- and deceased-donor transplantation is likely not due to difference in either DSA strength (Table S7a) nor in immunization status, as there was no stronger effect of DSA on graft survival in patients retransplanted with a living donor kidney, than in patients receiving a living-donor kidney as first transplant with a much lower level of immunization (Table S8; Figure S3; Figure S4). However, it is known that graft survival rates of poorly HLA matched living-donor grafts are superior to those of well HLA matched deceased-donor grafts (16,32). This can partially be explained by the prolonged cold ischemia time in deceased-donors, but also inherent factors of the organ due to cardiovascular instability of the donor prior to nephrectomy may play a role (33). Donor kidney quality is of importance when transplanting patients with DSA. Relevant variables regarding kidney quality are for example donor age, (deceased) donor type, CIT and graft function which are all available in our database. We corrected in our multivariable analysis analyzing the effect of pretransplant DSA on graft survival for donor age, donor type and CIT (CIT only in deceased-donor transplantation), but not for (delayed) graft function as this might be caused by the pre-existent DSA.

Inter-individual differences in the level of HLA antigen expression on the cell surface have been shown to impact the CDC-XM (34), indicating that sufficient HLA expression is required to induce effector mechanisms such as complement activation. The limited impact of preformed DSA against HLA class-I or class-II in living-donor transplantation compared to deceased-donor transplantation might be explained by a lower expression of HLA and adhesion molecules on the endothelial cells in living-donor organs compared to those of deceased-donors (35). In our cohort of 1487 living-donor kidney transplantations the combination of class-I and -II antibodies occurred in only 18 cases indicating that the prevalence of this risk factor is relatively low.

In conclusion, our study demonstrated that in the presence of a negative CDC-XM, SAB-defined DSA against either HLA class-I or HLA class-II are a significant risk factor in deceased-donor transplantation, but this seems not to be the case in living-donor transplantation. The combined presence of DSA against HLA class-I and -II has a much stronger negative impact on graft survival after both deceased-donor transplantation, while in living-donor transplantations class-I and -II DSA seem to be associated with an increased risk for graft failure but this could not be assessed due to their low prevalence. Based on these results, we suggest that the combination of class-I and -II DSA is taken into account in the allocation of donor kidneys in Eurotransplant region. Moreover, recipients with this combination of DSA should be considered as patients with a higher risk of graft failure.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

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Figure legends

Figure 1. Prevalence of pretransplant HLA-Abs and DSA in the total cohort (N=4724).

(A) Venn diagram showing the prevalence of pretransplant HLA-A/B/DR/DQ HLA-Abs.

(B) Venn diagram showing the prevalence of pretransplant HLA-A/B/DR/DQ DSA.

Figure 2. Long-term graft survival of kidney transplants according to the presence of pretransplant DSA.

(A) Adjusted Kaplan-Meier estimates (AKME) for death-censored graft survival according to the presence of pretransplant DSA for the total cohort including deceased and living-donor transplantations (N=4724).

(B) AKME for death-censored graft survival according to the presence of pretransplant DSA for living-donor transplantations only (N=1487).

(C) AKME for death-censored graft survival according to the presence of pretransplant DSA for deceased-donor transplantations only (N=3237).

All AKME were adjusted for the same covariates: recipient age (quadratic) and donor age (quadratic), donor type (living or deceased; for the total cohort only), cold ischemia time (for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic) and induction therapy with IL-2 receptor blocker.

Figure 3. Impact of DSA on graft survival for deceased-donor transplantations.

(A) Adjusted Kaplan-Meier estimates (AKME) for 1-year death-censored graft survival according to the presence of pretransplant DSA for living-donor transplantations only (N=1487).

(B) AKME for 1-year death-censored graft survival according to the presence of pretransplant DSA for deceased-donor transplantations only (N=3237).

(C) Analysis of long-term effect of pretransplant DSA starting at 1 year after transplantation for living-donor transplantations only (N=1417).

(D) Analysis of long-term effect of pretransplant DSA starting at 1 year after transplantation for deceased-donor transplantations only (N=2834).

(E) AKME for death-censored graft survival according to the presence of pretransplant HLA class-I (A/B) and/or -II (DR/DQ) DSA for living-donor transplantations only (N=1487).

(F) AKME for death-censored graft survival according to the presence of pretransplant HLA class-I (A/B) and/or -II (DR/DQ) DSA for deceased-donor transplantations only (N=3237).

All AKME were adjusted for the same covariates: recipient age (quadratic) and donor age (quadratic), donor type (living or deceased; for the total cohort only), cold ischemia time (for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic) and induction therapy with IL-2 receptor blocker.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Supplemental Materials

Figure S1. Long-term graft survival of kidney transplants according to the presence of pretransplant DSA.

Figure S2. Long-term graft survival of kidney transplants according to the presence of pretransplant DSA for different MFI cutoffs.

Figure S3. Long-term graft survival of first (black line) versus retransplants (red line) of DSA positive living-donor kidney transplantations.

Figure S4. Kaplan-Meier death censored graft survival for first (black) vs. retransplants (blue). Left: no HLA-antibodies detected in pre-Tx serum, middle: not donor specific HLA-antibodies (NDSA) detected, right: donor specific HLA antibodies detected. Top panel total cohort (N=4724), middle panel living donors (N=1487) and bottom panel deceased donors (N=3237).

Table S1. Prevalence of HLA-Abs only within total cohort (N=4724).

Table S2. Patient, donor and transplant characteristics for transplantations with pretransplant DSA stratified according to donor type.

Table S3. Patient, donor and transplant characteristics for transplantations with pretransplant DSA class I only stratified according to donor type.

Table S4. Patient, donor and transplant characteristics for transplantations with pretransplant DSA class II only stratified according to donor type.

Table S5. Patient, donor and transplant characteristics for transplantations with pretransplant DSA class I and II stratified according to donor type.

Table S6. Multivariable analyses of DSA using Cox proportional hazards model including the Hazard ratios for all parameters used in the model.

Table S7. (A) DSA strength (Number of DSA, Maximum MFI of DSA, Cumulative MFI of DSA) in living- versus deceased-donor transplantations. (B) DSA strength (Number of DSA, Maximum MFI of DSA, Cumulative MFI of DSA) for DSA Class I only, Class II only and Class I and II. (C) DSA strength (Number of DSA, Maximum MFI of DSA, Cumulative MFI of DSA) for DSA Class I only, Class II only and Class I

and II in deceased-donor transplantations. (D) DSA strength (Number of DSA, Maximum MFI of DSA, Cumulative MFI of DSA) for DSA Class I only, Class II only and Class I and II in living-donor transplantations.

Table S8. Percentage panel reactive antibodies (at time of transplantation and in peak serum) for DSA positive living-donor transplantations.

Table S9. Average number of split level HLA mismatches between recipient and donor per HLA locus, DSA vs no DSA for both living- and deceased-donor transplantations.

Table 1. Patient, donor and transplant characteristics.

Characteristics	No DSA (N = 4157)	DSA (N = 567)	p-value	Total cohort (N = 4724)
Patient				
Age at transplantation (years, mean ± SD)	45.6 ± 14.4	44.2 ± 13.9	0.01 [a]	45.4 ± 14.4
Female sex - no. (%)	1561 (37.6)	333 (58.7)	<0.001 [b]	1894 (40.1)
PRA at time of transplantation (%, mean ± SD)	3.5 ± 12.9	24.4 ± 30.7	<0.001 [a]	6.0 ±17.5
Highest PRA (%, mean ± SD)	9.8 ± 21.0	43.6 ± 36.3	<0.001 [a]	13.8 ± 25.8
Dialysis – no. (%)			0.0004 [b]	
No	472 (1.4)	43 (7.6)		515 (10.9)
Yes – hemodialysis	2140 (51.4)	332 (58.6)		2472 (52.3)
Yes – peritoneal dialysis	1529 (36.8)	186 (32.8)		1715 (36.3)
Unknown	16 (0.4)	6 (1.1)		22 (0.5)
Time on dialysis (years, mean ± SD)	2.7 ± 2.4	3.4 ± 3.0	<0.0001 [a]	2.8 ± 2.5
Donor				
Donor Age (years, mean ± SD)	44.4 ± 14.9	44.0 ± 15.8	1.00 [a]	44.4 ± 15.0
Donor Female Sex - no. (%)	2128 (51.2)	258 (45.5)	0.01 [b]	2386 (50.5)
Type of donor - no (%)			<0.001 [b]	
Living	1350 (32.5)	137 (24.2)		1487 (31.4)
Deceased – DBD	2076 (49.9)	351 (61.9)		2427 (51.4)
Deceased – DCD	731 (17.6)	79 (13.9)		810 (17.2)
Cold-ischemia time (hours, mean ± SD)				
Deceased-donors	21.7 ± 7.3	22.8 ± 6.8	0.001 [a]	21.9 ± 7.2
Living-donors	2.5 ± 1.6	2.5 ± 1.0	0.53 [a]	2.5 ± 1.5
Transplant				
Retransplantation - no. (%)	453 (10.9)	270 (47.6)	<0.001 [b]	723 (15.3)
HLA-A/B/DR broad mismatches (mean ± SD)	2.3 ± 1.5	2.4 ± 1.3	0.15 [a]	2.4 ± 1.5
Induction Therapy				
IL-2 receptor blocker - no. (%)	913 (21.9)	109 (19.2)	0.14 [b]	1022 (21.6)
T-cell depleting antibody * - no. (%)	145 (3.6)	39 (6.9)	<0.001 [b]	184 (3.9)

Initial immunosuppression - no. (%)				
Steroids	4069 (97.9)	547 (96.5)	0.04 [b]	4616 (97.7)
MMF / Azathioprine	3163 (76.1)	442 (78)	0.20 [b]	3605 (76.3)
Cyclosporine / Tacrolimus	3892 (93.6)	542 (95.6)	0.07 [b]	4434 (93.9)
Sirolimus	260 (6.3)	26 (4.6)	0.12 [b]	286 (6.1)
Other	555 (13.4)	53 (9.4)	0.008 [b]	608 (12.9)
Unknown	14 (0.3)	3 (0.5)	0.47 [b]	17 (0.4)

[a] Mann-Whitney U test for continuous variables; [b] Chi-square test for categorical variables.
* T-cell depleting antibody therapy: ALG, ATG, OKT3 MoAb.
DBD: donation after brain death, DCD: donation after cardiac death, DSA: donor-specific HLA antibodies, MMF: mycophenolate mofetil, PRA: Panel reactive antibodies.

Table 2. Multivariable analyses of DSA using Cox proportional hazards model.

	No. (%) of Transplants with DSA	Hazard Ratio DSA	95% CI
Total cohort (N=4724)	567 (12%)	1.77	1.51 - 2.08
Living donors (N=1487)			
All	137 (9%)	1.42	0.95 - 2.10
DSA Class-I only	58 (4%)	1.46	0.83 - 2.55
DSA Class-II only	61 (4%)	1.17	0.64 - 2.14
DSA Class-I and -II	18 (1%)	2.84	1.05 - 7.69
Early failures (<1Y)	137 (9%)	1.69	0.76 - 3.77
Late failures (≥1Y)	128 (9%)	1.35	0.86 - 2.12
Deceased donors (N=3237)			
All	430 (13%)	1.86	1.56 - 2.21
DSA Class-I only	182 (6%)	1.93	1.50 - 2.47
DSA Class-II only	187 (6%)	1.76	1.37 - 2.26
DSA Class-I and -II	61 (2%)	1.96	1.29 - 2.98
Early failures (<1Y)	430 (13%)	1.76	1.33 - 2.33
Late failures (≥1Y)	352 (12%)	1.97	1.56 - 2.45

CI: Confidence interval, DSA: donor-specific HLA antibodies. In this multivariable analysis we adjusted for differences in the following covariates: recipient age (quadratic), donor age (quadratic), donor type (living or deceased), cold ischemia time in hours for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic) and induction therapy with IL-2 receptor blocking antibody. The Hazard ratios of the covariates are shown in Table S6.

Table 3. Patient, donor and transplant characteristics for deceased and living donor transplantations

Characteristics	Deceased Donor (N = 3237)	Living Donor (N = 1487)	p-value	Total cohort (N = 4724)
Patient				
Age at transplantation (years, mean ± SD)	46.9 ± 14.1	42.3 ± 14.5	<0.001 [a]	45.4 ± 14.4
Female sex - no. (%)	1309 (40.4)	585 (39.4)	0.47 [b]	1894 (40.1)
PRA at time of transplantation (%, mean ± SD)	7.0 ± 19.0	3.8 ± 13.4	<0.001 [a]	6.0 ±17.5
Highest PRA (%, mean ± SD)	16.5 ± 28.3	8.0 ± 17.9	<0.001 [a]	13.8 ± 25.8
Dialysis – no. (%)			<0.001 [b]	
No	150 (4.6)	365 (24.6)		515 (10.9)
Yes – hemodialysis	1879 (58.1)	593 (39.9)		2472 (52.3)
Yes – peritoneal dialysis	1189 (36.7)	526 (35.4)		1715 (36.3)
Unknown	19 (0.6)	3 (0.2)		22 (0.5)
Time on dialysis (years, mean ± SD)	3.4 ± 2.6	1.3 ± 1.5	<0.001 [a]	2.8 ± 2.5
Donor				
Donor Age (years, mean ± SD)	42.8 ± 16.0	47.9 ± 11.9	<0.001 [a]	44.4 ± 15.0
Donor Female Sex - no. (%)	1517 (47.9)	869 (58.4)	<0.001 [b]	2386 (50.5)
Cold-ischemia time (hours, mean ± SD)	21.9 ± 7.2	2.5 ± 1.5	<0.001 [a]	15.8 ± 10.8
Transplant				
Retransplantation - no. (%)	562 (17.4)	161 (10.8)	<0.001 [b]	723 (15.3)
HLA-A/B/DR broad mismatches (mean ± SD)	2.2 ± 1.4	2.7 ± 1.6	<0.001 [a]	2.4 ± 1.5
Induction Therapy				
IL-2 receptor blocker - no. (%)	655 (20.2)	367 (24.7)	<0.001 [b]	1022 (21.6)
T-cell depleting antibody * - no. (%)	133 (4.1)	51 (3.4)	0.26 [b]	184 (3.9)
Initial immunosuppression - no. (%)				
Steroids	3172 (98.0)	1444 (97.1)	0.058 [b]	4616 (97.7)
MMF / Azathioprine	3163 (76.1)	442 (78)	<0.001 [b]	3605 (76.3)
Cyclosporine / Tacrolimus	3051 (94.3)	1383 (93.0)	0.097 [b]	4434 (93.9)
Sirolimus	176 (5.4)	110 (7.4)	<0.001 [b]	286 (6.1)
Other	436 (13.5)	172 (11.6)	0.070 [b]	608 (12.9)
Unknown	11 (0.3)	6 (0.4)	0.73 [b]	17 (0.4)

[a] Mann-Whitney U test for continuous variables; [b] Chi-square test for categorical variables.

* T-cell depleting antibody therapy: ALG, ATG, OKT3 MoAb.

DBD: donation after brain death, DCD: donation after cardiac death, DSA: donor-specific HLA antibodies, MMF: mycophenolate mofetil, PRA: Panel reactive antibodies.

Figure 1

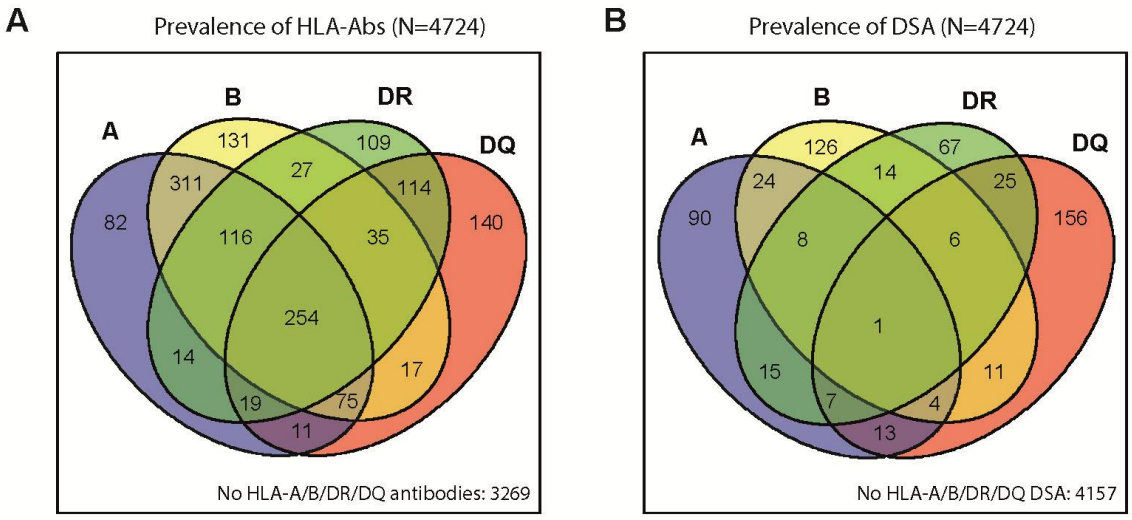

Figure 2

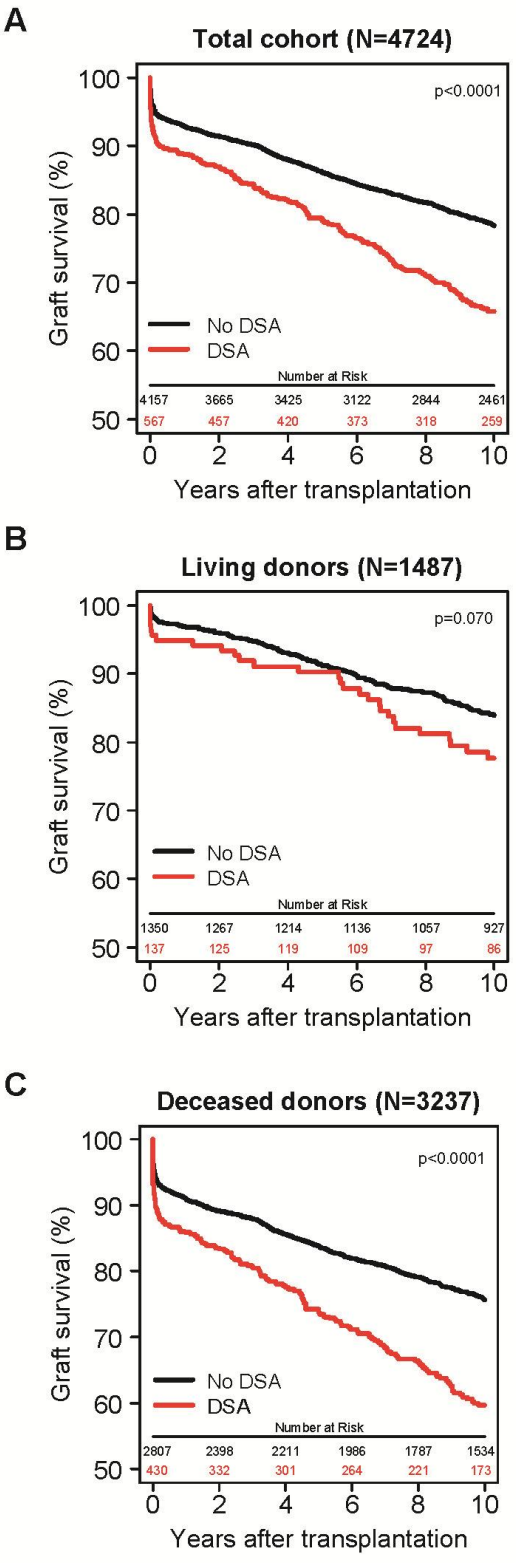

Figure 3